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(54) Title: SERUM ANTIOXIDANTS AS PREDICTORS OF ADULT RESPIRATORY DISTRESS SYNDROME

(57) Abstract

At the initial diagnosis of sepsis (6-24h before the development of ARDS), serum manganese superoxide dismutase (MnSOD) levels and catalase activities are increased in septic patients who subsequently developed ARDS compared to septic patients who did not develop ARDS. Increases in MnSOD and catalase may be used to predict the occurrence of ARDS in septic patients with the same sensitivity, specificity and efficiency as parallel assessments of serum lactate dehydrogenase (LDH) and Factor VIII levels. Evaluation of serum MnSOD and catalase as well as these other accessible markers facilitates identification of subsets of patients and allow prospective treatment of septic patients who are destined to develop ARDS.

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SERUM ANTIOXIDANTS AS PREDICTORS OF ADULT RESPIRATORY DISTRESS SYNDROME

BACKGROUND

The present application relates in general to methods and apparatus for performing assays for disease states, and in particular to methods and apparatus for performing assays for adult respiratory distress syndrome (ARDS).

ARDS is an acute inflammatory process characterized by lung neutrophil accumulation, lung edema and progressive hypoxemia [Repine, *Lancet*, *339*, 466-469 (1992)]. ARDS occurs as a complicating factor in patients with sepsis as well as numerous other predisposing conditions. Since many common and diverse risk factors lead to the development of ARDS, but ARDS develops only relatively rarely, pretreating everyone at risk for ARDS is not practical [Fowler et al., *Ann. Intern. Med.*, *98*, 593-597 (1983)]. Because a better understanding of ARDS is emerging and various interventions which can limit inflammation are forthcoming, it has become a major goal to identify accessible and repeatable markers in at risk patients which predict the development of ARDS. This will enable experimental therapies to be prospectively and effectively evaluated in smaller, better-defined groups of patients.

SUMMARY OF THE INVENTION

The present invention provides a method for identifying septic patients for prospective treatment of adult respiratory distress syndrome including the step of determining a high (greater than an established baseline) serum level of manganese superoxide dismutase.

The present invention also provides a method for identifying septic patients for prospective treatment of adult respiratory distress syndrome including the step of determining a high (greater than an established baseline) serum level of catalase.

According to the present invention, apparatus for identifying septic patients for prospective treatment of adult respiratory distress syndrome includes means for determining a high (greater than an established baseline) serum level of manganese superoxide dismutase.

The present invention also provides apparatus for identifying septic patients for prospective treatment of adult respiratory distress syndrome including means for determining a high (greater than an established baseline) serum level of catalase.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of MnSOD levels for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times: at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3);

FIG. 2 is a graph of CAT activity for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times: at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3);

FIG. 3 is a graph of GPX activity for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times: at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3);

FIG. 4 is a graph of LDH activity for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times: at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3);

FIG. 5 is a graph of Factor VIII levels for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times: at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3); and

FIG. 6 is a graph of levels of α_1Pi-elastase complexes for healthy control subjects (squares); and of septic patients who did not develop ARDS (triangles) and septic patients who developed ARDS (circles) at three times: at diagnosis of sepsis (1), at diagnosis of ARDS (2) and after diagnosis of ARDS (3).

In the experiments illustrated in FIGs. 1-6, septic patients were enrolled (0 h) and studied sequentially for the next 48 h. Points were plotted at the diagnosis of sepsis (0 h at 1), at the diagnosis of ARDS (6-24 h after the diagnosis of sepsis at 2) and after the diagnosis of sepsis (6-24 h after the diagnosis of ARDS at 3). Each value is the mean ± SE of 3-20 determinations.

DETAILED DESCRIPTION OF THE INVENTION

In the present investigation, three antioxidant enzymes [manganese superoxide dismutase (MnSOD), catalase and glutathione peroxidase (GPX)] were compared with three other potential markers [Factor VIII [Carvalho et al., *N. Engl. J. Med., 307,* 1113-1119 (1982) and Rubin et al., *J. Clin. Invest., 86,* 474-480 (1990)] LDH [Ward et al., *J. Clin. Invest.* 76, 517-527 (1985) and Dwenger et al., In: Sturm, ed. *Adult Respiratory Distress Syndrome,* Berlin Heidelberg: Springer-Verlag, 91-127 (1991)]] and α_1Pi-elastase complexes [Rocker et al., *Lancet, 1,* 120-123 (1989) and Hilgenfeldt et al., *Eur. J. Clin. Pharmacol, 38,* 125-131 (1990)] for their ability to predict the development of ARDS in patients with sepsis.

Alterations occur in the oxidant-antioxidant balance in ARDS and in other disease states that appear to involve oxygen radicals in their pathogenesis [Leff et al., *Free Radical Biol. Med., 13,* 143-149 (1992); Leff et al., *Am. Rev. Respir. Dis, 146,* 985-989 (1992); Bühl et al., *Lancet, 2,* 1294-1298 (1989); Bernard et al., *Am. Rev. Resp. Dis., 139,* A221 (Abstract) (1989); and Pacht et al., *Chest, 100,* 1397-1403 (1991)]. In addition, patients with established ARDS have

elevated serum catalase activity [Leff et al., *Am. Rev. Respir. Dis, 146,* 985-989 (1992)]. Serum catalase activity increased in a rat model of burn-induced acute lung injury [Leff et al., *Inflammation* (In Press) (1992)].

EXAMPLE

Patient Consent and Selection. After written consent was obtained from the patient or a family member, each subject was studied using a protocol which was approved by an institutional human subjects review committee. All patients (n=26) who were identified within 8 h of the diagnosis of sepsis were eligible for enrollment. Patients with sepsis had a serious bacterial infection and either (a) a rectal or core temperature exceeding 39°C or (b) a peripheral leukocyte count of >12,000 cells/mm^3 or >20% immature neutrophils. Septic patients also had at least one of the following: a positive blood culture involving a commonly accepted pathogen, a strongly suspected or proven source of systemic infection, gross pus in a closed space, unexplained systemic arterial hypotension (systolic blood pressure less than 80 mm Hg), systemic vascular resistance less than 800 dyn x s x cm^2 and/or unexplained metabolic acidosis [Parsons et al., *Am. Rev. Resp. Dis., 140,* 294-301 (1989)].

Patients with ARDS (n=6) met the following criteria: (1) acute respiratory failure requiring mechanical ventilation, (2) bilateral pulmonary infiltrates, (3) pulmonary capillary wedge pressure <18 mm Hg, (4) static pulmonary compliance <50 ml/cm H_2O, and (5) arterial to alveolar partial pressure of oxygen ratio of <0.25 [Parsons et al., *Am. Rev.*

Resp. Dis., 140, 294-301 (1989)]. Serum and plasma samples were obtained at the diagnosis of sepsis (0 h) and at the diagnosis of ARDS (6-24 h after the diagnosis of sepsis) and after the diagnosis of ARDS (6-24 h after the diagnosis of ARDS) either through an indwelling arterial or venous catheter or by direct venipuncture. Patients were divided into two groups: septic patients who did not develop ARDS and septic patients who later developed ARDS. Patients were prospectively and sequentially studied until death or discharge. All assays were performed by personnel who were unaware of the diagnoses. Control subjects (n=15) were healthy individuals.

Source of reagents. Hanks' balanced salt solution (HBSS) was purchased from Gibco Laboratories (Grand Island, New York). All other reagents were obtained from Sigma Chemical Company (St. Louis, Missouri).

Measurement of serum markers. MnSOD [Kawaguchi et al., *Biochem. Biophys. Res. Commun. 171,* 1378-1386 (1990)], Factor VIII antigen [Cejka, *Clin. Chem., 28(6),* 1356-1358 (1982)] and α_1Pi-elastase complexes [Duswald et al., *Surgery, 98,* 892-899 (1985)] were measured by ELISA. Catalase was assessed by polarographic assessment of O_2 evolution [Leff et al., *J. Appl. Physiol., 71(5),* 1903-1906 (1991)]. GPX was measured as the oxidation of NADPH at 340 nm in glutathione reductase, glutathione and t-butyl hydroperoxide [Beutler, *A Manual of Biochemical Methods,* Orlando, Grune & Stratton, Inc., 1-172 (1984)], LDH [Beutler, *A Manual of Biochemical Methods,* Orlando, Grune & Stratton, Inc., 1-172 (1984)] and albumin [Corcoran et al., *Clin. Chem., 23,* 765-766 (1977)] were assayed spectrophotometrically. Uric acid was

measured by HPLC [Terada et al., *J. Appl. Physiol., 65,* 2349-2353 (1988)].

Statistical analyses. Patient groups were compared using an analysis of variance with a Student-Newman-Keuls test of multiple comparisons. An unpaired t test was used to compare the clinical characteristics of septic patients with or without ARDS. For calculations of sensitivity, specificity, positive or negative predictive values and efficiency, 95% confidence intervals were determined based on the binomial distribution [Cochran, *In: Sampling Techniques,* 2nd ed., New York, John Wiley & Sons, Inc., 54-59 (1963)]. Significance was accepted at a p value of <0.05.

Clinical Parameters. Septic patients who subsequently developed ARDS and septic patients who did not develop ARDS were the same ($p>0.05$) with respect to age, gender, hematocrit, hemoglobin, blood leukocyte count, blood neutrophil count, serum SGOT, bilirubin, albumin, uric acid levels and APACHE II score [Leff et al., *Ann. Rev. Respir. Dis., 146,* 985-989 (1992); Knaus et al., *Crit. Care Med., 13,* 818-289 (1985)]. The mortality of septic patients who developed ARDS was 50% (3 of 6) compared to a mortality of 30% (6 of 20) in septic patients who did not develop ARDS.

Blood markers patterns. Septic patients had increased ($p<0.05$) serum MnSOD levels compared to control subjects (FIG. 1). However, at the initial diagnosis of sepsis (approximately 6-24 h before diagnosis of ARDS), septic patients who eventually developed ARDS had increased ($p<0.05$) serum MnSOD levels compared to septic patients who did not develop ARDS. Serum MnSOD levels remained elevated for the next 48 h in patients who developed

ARDS while MnSOD levels returned to control levels during the next 48 h in septic patients who did not develop ARDS.

Similarly, at the diagnosis of sepsis, serum from septic patients had more ($p<0.05$) catalase activity than serum from control subjects. Again, at the initial diagnosis of sepsis, patients who later developed ARDS had more ($p<0.05$) serum catalase activity than septic patients who did not develop ARDS (FIG. 2). During the next 48 h, serum catalase activity increased progressively in septic patients who developed ARDS but did not change in septic patients who did not develop ARDS.

In contrast to MnSOD levels and catalase activities, serum GPX activity was essentially the same ($p>0.05$) in control subjects and septic patients regardless of whether ARDS ensued (FIG. 3).

Serum from septic patients who subsequently developed ARDS also had increased ($p<0.05$) LDH activity compared to serum from septic patients who did not develop ARDS. Serum from septic patients who did not develop ARDS had the same ($p>0.05$) LDH activity as serum from control subjects (FIG. 4). Serum LDH measurements increased during the 48 h study period in septic patients who developed ARDS but not in septic patients who did not develop ARDS.

Septic patients who did or did not develop ARDS (FIG. 5) had similarly increased ($p<0.05$) serum Factor VIII levels compared to control subjects. Septic patients who did and did not develop ARDS had similar ($p>0.05$) Factor VIII levels.

Finally, plasma α_1Pi-elastase complexes were increased in all septic patients at the initial diagnosis of sepsis but differences between septic

patients who did or did not develop ARDS were manifest only at the time of diagnosis of ARDS (6-24h after the diagnosis of sepsis) (FIG 6). By 48 h after the initial diagnosis of sepsis, α_1Pi-elastase complexes had similarly decreased in septic patients independent of the development of ARDS.

Analyses of serum markers. First, no correlations were found at any time between any of the six markers; Second, the positive and negative predictive values and the sensitivity and specificity of Serum MnSOD levels (≥ 450 ng/ml), catalase activity (≥ 30 U/ml), LDH activity ≥ 250 U/L and Factor VIII levels ≥ 445% control were comparable in predicting the development of ARDS in septic patients (Table 1). Third, serum MnSOD levels, catalase and LDH activity exceeded 450 ng/ml, 30 U/ml and 250 U/L, respectively, approximately 9 h, 12 h and 12 h, on average, respectively, before the diagnosis of ARDS. Further results appear in Table 1.

TABLE 1

Comparison of the Sensitivity and Specificity of Blood Markers as Predictors of ARDS in Septic Patients

Parameter	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Efficiency
MnSOD ≥ 450 ng/ml	67% (42-94)	88% (75-98)	67% (4-94)	88% (75-98)	83% (70-94)
Catalase ≥ 30 U/ml	83% (61-99)	65% (49-82)	42% (25-68)	93% (81-100)	69% (55-84)
GPX ≥ 0.72 U/ml	50% (27-85)	47% (31-69)	25% (12-53)	73% (53-92)	48% (34-66)
LDH ≥ 250 U/L	67% (42-94)	78% (62-92)	50% (29-81)	88% (74-98)	75% (61-89)
Factor VIII ≥ 445% Control	83% (61-99)	67% (42-94)	45% (27-73)	92% (80-100)	71% (57-85)
α_1Pi-elastase >940ng/ml	67% (37-98)	64% (50-80)	18% (8-47)	94% (84-100)	64% (51-79)

In Table 1 each value represents 3-20 determinations at study entry (t=0 h). Values in parentheses represent 95% confidence intervals. Also in Table 1; Sensitivity = TP/TP + FN; Specificity = TN/TN + FP; Positive Predictive Value = TP/TP + FP; Negative Predictive Value = TN/TN + FN; and Efficiency = TP + TN/TP + FP + TN + FN.

In Table 1, results are shown for six sequentially measured factors in the blood of septic patients who were predisposed to develop ARDS. Nine to twelve hours before the development of ARDS, two serum antioxidant enzymes, MnSOD and catalase, were increased in septic patients who later developed ARDS compared to septic patients who did not develop ARDS and that both of these factors predicted the development of ARDS in septic patients with as good a sensitivity, specificity and efficiency as measurements of LDH and Factor VIII. By comparison, measurements of GPX and α_1Pi-elastase complexes were neither different in septic patients who did or did not subsequently develop ARDS nor effective in predicting the development of ARDS in septic patients.

Assessment of MnSOD and catalase are useful for defining the pathogenesis of ARDS or identifying patients with similar pathophysiologies. Each measurement is accessible, repeatable and relatively easy to perform. Based on assessment of these markers, study of prophylactic treatment is facilitated by reducing the number of at risk individuals who need to be studied to obtain patients with ARDS.

Increases in serum MnSOD levels and serum catalase activity may also have functional importance. MnSOD and catalase may diminish oxidant

insults mediated by superoxide anion($O_2^-\cdot$) or hydrogen peroxide (H_2O_2) or their products such as hydroxyl radical ($\cdot OH$). This possibility may be especially relevant because accelerated intravascular generation of oxygen radicals from stimulated neutrophils, circulating xanthine oxidase or other sources are implicated in the pathogenesis of sepsis and ARDS [McGuire et al., *J. Clin. Invest., 69,* 543-553 (1982); Cochrane et al., *J. Clin. Invest. 71,* 754-758; (1983); Baldwin et al., *Lancet, 1,* 11-14 (1986) and Grum et al., *J. Crit. Care, 2,* 22-26 (1987)].

Because the patterns were different for various markers and no two markers correlated with each other, each factor may represent a distinct process and these factors may more correctly reflect various processes occurring in septic patients with ARDS rather than ARDS *per se*. The present work has focused on sepsis-induced ARDS, so different mechanisms may be present in patients who develop ARDS following trauma and other predispositions.

The origins of the factors, although unclear, most likely are multiple. Lung tissue injury is a possible source for increases in LDH, MnSOD, catalase and Factor VIII levels. Endothelial cells are rich in these factors and, if perturbed, may readily increase the levels of these factors in the blood. However, intravascular neutrophil activation may be responsible for increases in α_1Pi-elastase complexes because elastase may be present only in neutrophils. Notably, increases in α_1Pi-elastase complexes occurred relatively later, at the diagnosis of ARDS, and then decreased by 48 h after the diagnosis of sepsis, which may indicate a decline in neutrophil activity. Red blood cell

(RBC) hemolysis may be a source for increases in serum catalase and LDH activity, but not MnSOD or Factor VIII levels may not, because RBCs do not contain the latter. Serum catalase activity is also increased in the serum of rats subjected to skin burn [Leff et al., *Inflammation* (In Press) (1992)], and patients with the acquired immunodeficiency syndrome [Leff et al., *Am. Rev. Respir. Dis, 146,* 985-989 (1992)], but again, in these situations, the source is unclear. Elevations of IL-1, tumor necrosis factor (TNF) and endotoxin have been found in ARDS patients [Parsons et al., *Am. Rev. Resp. Dis., 140,* 294-301 (1989); Suter et al., *Am. Rev. Resp. Dis., 145,* 1016-1022 (1992); Siler et al., *Exp. Lung Res., 15(6),* 881-894 (1989); Hyers et al., *Am. Rev. Respir. Dis., 144,* 268-271 (1991) and Marks et al., *Am. Rev. Resp. Dis., 141,* 94-97 (1990)] and may cause increases in antioxidants such as MnSOD and catalase [White et al., *J. Appl. Physiol., 66,* 1003-1007 (1989); Wong et al., *Science, 242,* 941-944 (1988); Brown et al., *Proc. Natl. Acad. Sci. (USA), 86,* 2516-2520 (1989) and Taniguchi, *Adv. Clin. Chem., 29,* 1-59 (1992)].

Although the present invention is illustrated by the above embodiments, it is expected that variations and modifications will occur to those skilled in the art upon consideration of the present disclosure. Accordingly, it is intended that the present invention include all modifications and variations which come within the scope of the claims.

WHAT IS CLAIMED IS:

1. A method for prospectively identifying septic patients for treatment of adult respiratory distress syndrome comprising the steps of determining a septic patient's serum level of manganese superoxide dismutase, comparing the septic patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, said baseline serum level being predictive of the development of ARDS in septic patients, and identifying a septic patient whose serum level of manganese superoxide dismutase is predictive of the development of adult respiratory distress syndrome.

2. The method of claim 1 further comprising the steps of determining a septic patient's serum level of catalase, comparing the septic patient's serum level of catalase to an established baseline serum level of catalase, said baseline serum level being predictive of the development of ARDS in septic patients, and identifying a septic patient whose serum level of catalase is predictive of the development of adult respiratory distress syndrome.

3. Apparatus for prospectively identifying septic patients for treatment of adult respiratory distress syndrome comprising means for determining a septic patient's serum level of manganese superoxide dismutase and means for comparing the septic patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, said baseline serum level being predictive of the development of ARDS in septic patients.

4. The apparatus of claim 3 further comprising means for determining a septic patient's serum level of catalase and means for comparing the septic patient's serum level of catalase to an established baseline serum level of catalase, said baseline serum level being predictive of the development of ARDS in septic patients.

5. The method of claim 1 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

6. The method of claim 2 wherein the patient's serum level of catalase is determined by assay of serum catalase enzymatic activity.

7. The apparatus of claim 3 wherein the means for determining a patient's serum level of manganese superoxide dismutase comprises an ELISA assay.

8. The apparatus of claim 4 wherein the means for determining a patient's serum level of catalase comprises an assay for catalase enzymatic activity.

9. A method for predicting the development of adult respiratory distress syndrome in septic patients comprising the steps of determining a patient's serum level of manganese superoxide dismutase, comparing the patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, said baseline serum level being predictive of the development of ARDS, and identifying a patient whose serum level of manganese superoxide dismutase is predictive of the development of adult respiratory distress syndrome.

10. The method of claim 9 further comprising the steps of determining a patient's serum level of catalase activity, comparing the patient's serum level of catalase activity to an established baseline serum level of catalase activity, said baseline serum level being predictive of the development of ARDS, and identifying a patient whose serum level of catalase activity is predictive of the development of adult respiratory distress syndrome.

11. An apparatus for predicting the development of adult respiratory distress syndrome in septic patients comprising means for determining a patient's serum level of manganese superoxide dismutase, and means for comparing the patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, said baseline serum level being predictive of the development of ARDS.

12. The apparatus of claim 11 further comprising means for determining a patient's serum level of catalase activity and means for comparing the patient's serum level of catalase activity to an established baseline serum level of catalase activity, said baseline serum level being predictive of the development of ARDS.

13. The method of claim 9 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

14. The apparatus of claim 11 wherein the means for determining a patient's serum level of manganese superoxide dismutase comprises an ELISA assay.

15. The use of an ELISA to determine the level of manganese superoxide dismutase in a sample for predicting the development of adult respiratory distress syndrome in septic patients.

16. A product for predicting the development of adult respiratory distress syndrome in septic patients, said product containing an ELISA for determining the level of manganese superoxide dismutase in a sample of the patient's serum.

17. A kit for predicting the development of adult respiratory distress syndrome in a septic patients using a sample of the patient's serum comprising:

a) a first monoclonal antibody having a high specific immuno-reactivity against human manganese superoxide dismutase, said first monoclonal antibody capable of forming a first immuno-reactive complex with manganese superoxide dismutase in the serum;

b) a second monoclonal antibody having a high specific immuno-reactivity against human manganese superoxide dismutase, said second monoclonal antibody being labelled with an enzyme and being capable of forming a second immuno-reactive complex with the first immuno-reactive complex; and

c) a substrate for the enzyme.

AMENDED CLAIMS

[received by the International Bureau on 7 September 1994 (07.09.94);
original claims 11,12 and 14 cancelled;
original claims 1-4,7-10,16 and 17 amended;
new claims 18-20 added;
other claims unchanged (5 pages)]

1. A method for prospectively identifying the development of adult respiratory distress syndrome (ARDS) comprising the steps of determining a septic patient's serum level of manganese superoxide dismutase, comparing said septic patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, a serum level greater than or equal to said baseline serum level being predictive of the development of ARDS in septic patients, and diagnosing ARDS development potential if said septic patient's serum level of manganese superoxide dismutase is greater than or equal to said established baseline serum level of manganese superoxide dismutase.

2. The method of claim 1 further comprising the steps of determining said septic patient's serum level of catalase, comparing said septic patient's serum level of catalase to an established baseline serum level of catalase, a serum level greater than or equal to said baseline serum level being predictive of the development of ARDS in septic patients, and diagnosing ARDS development potential if said septic patient's serum level of catalase is greater than or equal to said established baseline serum level of catalase.

3. An immunologic device for prospectively identifying the development of adult respiratory distress syndrome (ARDS) comprising: a means for immunologically determining a septic patient's serum level of manganese superoxide dismutase; and a means for comparing the septic patient's serum level of manganese superoxide dismutase to a baseline serum level of manganese superoxide dismutase, wherein a serum level of managanese superoxide dismatase greater than or equal to said baseline serum level is predictive of the development of ARDS in septic patients.

4. The device of claim 3 further comprising a means for determining a septic patient's serum level of catalase; and a means for comparing the septic patient's serum level of catalase to a baseline serum level of catalase, wherein a serum level of catalase greater than or equal to said baseline serum level is predictive of the development of ARDS in septic patients.

5. The method of claim 1 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

6. The method of claim 2 wherein the patient's serum level of catalase is determined by assay of serum catalase enzymatic activity.

7. The device of claim 3 wherein the means for determining a patient's serum level of manganese superoxide dismutase comprises an ELISA assay.

8. The device of claim 4 wherein the means for determining a patient's serum level of catalase comprises an assay for catalase enzymatic activity.

9. A method for predicting the development of adult respiratory distress syndrome (ARDS) in a septic patient comprising the steps of providing serum of said patient, determining said patient's serum level of manganese superoxide dismutase, comparing said patient's serum level of manganese superoxide dismutase to an established baseline serum level of manganese superoxide dismutase, a serum level greater than or equal to said baseline serum level being predictive of the development of ARDS, and predicting the development of ARDS if said patient's serum level of manganese superoxide dismutase is greater than or equal to said established baseline serum level of manganese superoxide dimutase.

10. The method of claim 9 further comprising the steps of determining said patient's serum level of catalase activity, comparing said patient's serum level of catalase activity to an established baseline serum level of catalase activity, a serum level greater than or equal to said baseline serum level being predictive of the development of ARDS, and predicting the development of ARDS if said patient's serum level of catalase activity is greater than or equal to said established baseline serum level of catalase activity.

13. The method of claim 9 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

15. The use of an ELISA to determine the level of manganese superoxide dismutase in a sample for predicting the development of adult respiratory distress syndrome in septic patients.

16. A product for predicting the development of adult respiratory distress syndrome in septic patients, said product including an ELISA for determining the level of manganese superoxide dismutase in a sample of the patient's serum, and a means for determining the level of catalase in a sample of the patient's serum.

17. A kit for predicting the development of adult respiratory distress syndrome in a septic patients using a sample of the patient's serum comprising:

a) a first monoclonal antibody having a high specific immuno-reactivity against human manganese superoxide dismutase, said first monoclonal antibody capable of forming a first immuno-reactive complex with manganese superoxide dismutase in the serum of a septic patient;

b) a second monoclonal antibody having a high specific immuno-reactivity against at least one of said first monoclonal antibody, said first immuno-reactive complex, and human manganese superoxide dismutase, said second monoclonal antibody being labelled with an enzyme and being capable of forming a second immuno-reactive complex; and

c) a substrate for the enzyme that is capable of producing a detectable signal in proportion to the amount of manganese superoxide dismutase in the second immuno-reactive complex.

18. The method of claim 9 wherein the patient's serum level of manganese superoxide dismutase is determined by ELISA.

19. The method of claim 9 wherein said established baseline serum level of manganese superoxide dismutase is 450 ng/ml.

20. The method of claim 10 wherein the established baseline serum level of catalase activity is 30 U/ml.

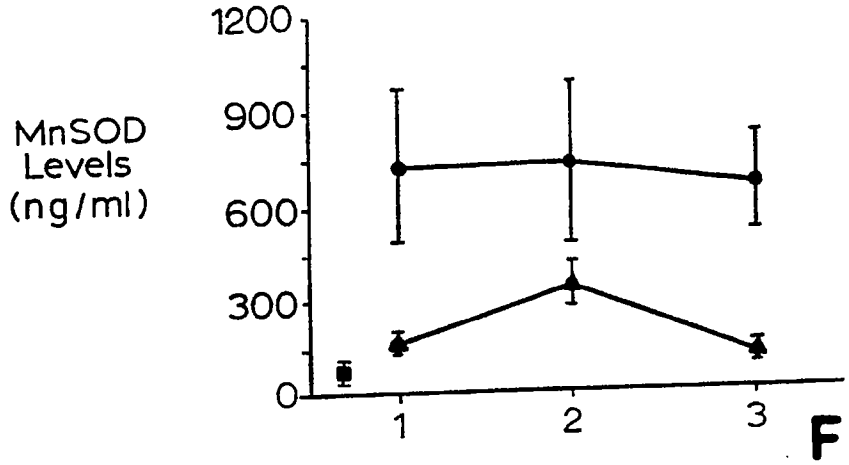

FIG. 1

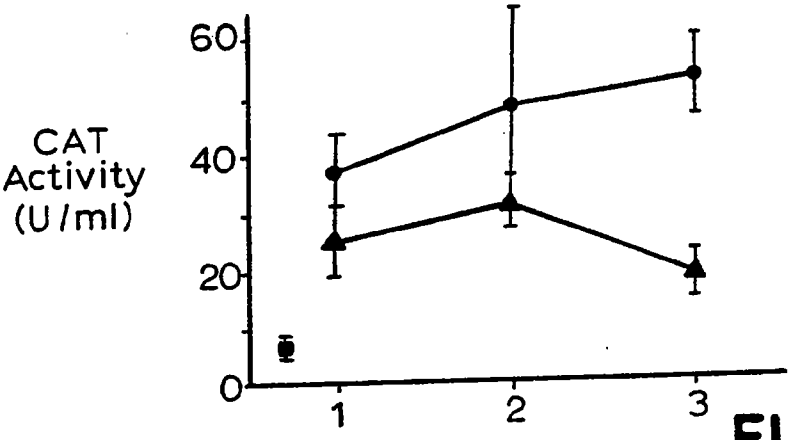

FIG. 2

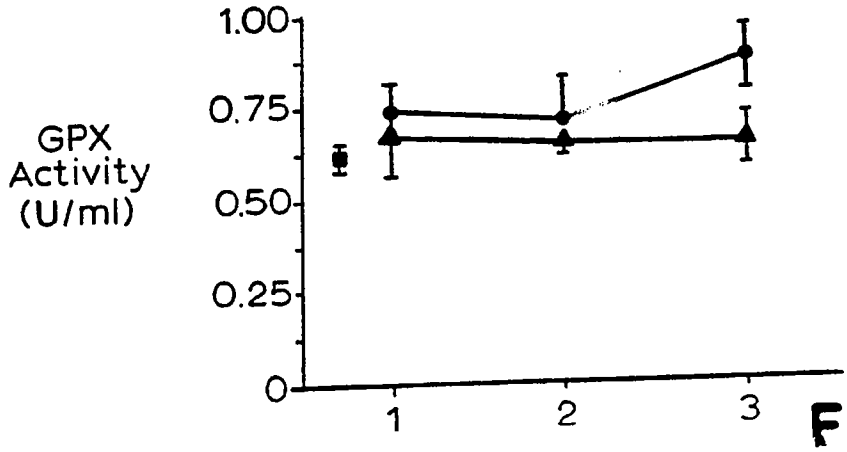

FIG. 3

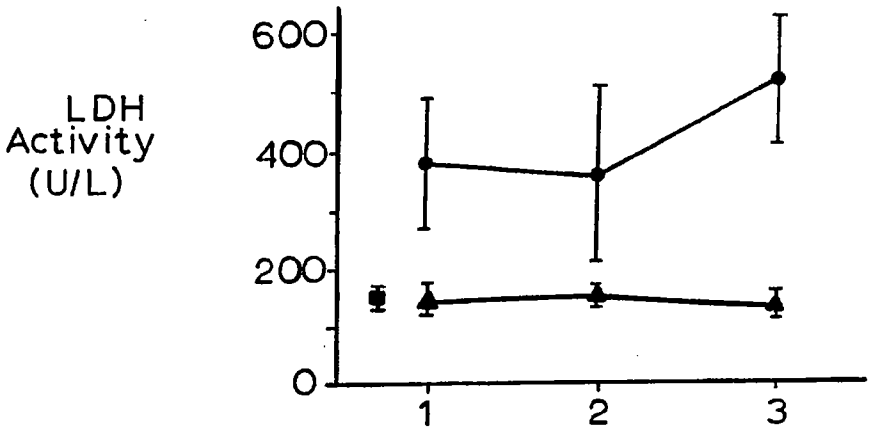

FIG.4

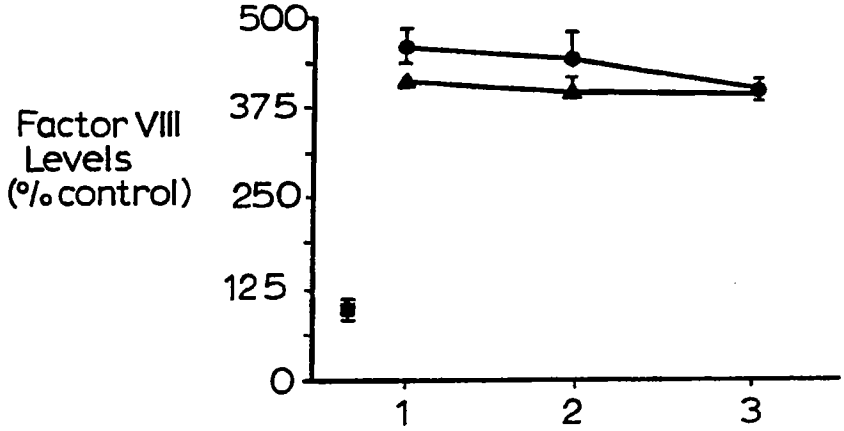

FIG. 5

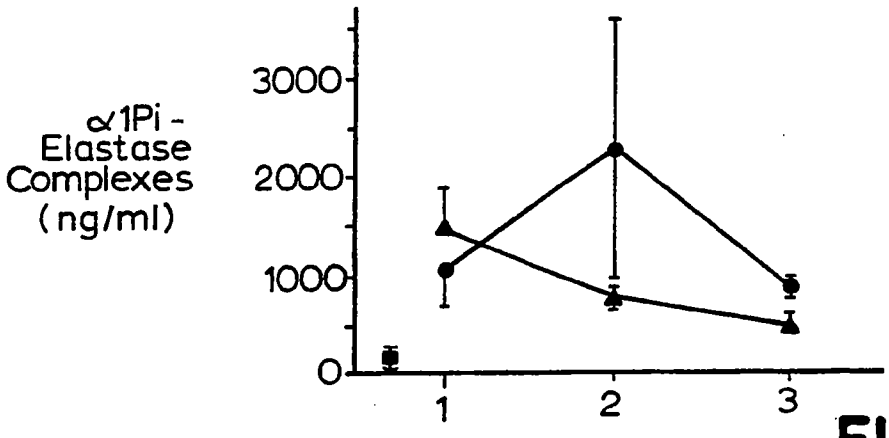

FIG. 6

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N33/573 C12Q1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLINICAL RESEARCH vol. 40, no. 1 , 1992 page 67A J.A.LEFF ET AL. 'Serum catalase activity is increased in septic patients and predictive of adult respiratory distress syndrome (ARDS) development.' see abstract ---	2,4,6,8, 10,12
X	AMERICAN REVIEW OF RESPIRATORY DISEASE vol. 143, no. 4(2) , April 1991 page A805 J.A.LEFF ET AL. 'Increased hydrogen peroxide scavenging and catalase activity in serum from septic patients who subsequently develop the adult respiratory distress syndrome (ARDS).' see abstract ---	2,4,6,8, 10,12

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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 1994	11. 07. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hitchen, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	THE LANCET vol. 341, no. 8848 , 27 March 1993 pages 777 - 780 J.A.LEFF ET AL. 'Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis.' see the whole document	1-17
A	SURGERY GYNECOLOGY AND OBSTETRICS vol. 167, no. 2 , August 1988 pages 92 - 98 R.J.BLOOM ET AL. 'Endotoxin and pulmonary cell injury' see abstract	1,2

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